A METHOD FOR THE DIFFERENTIAL STAINING OF FUNGOUS AND HOST CELLS

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In making histological studies of fungi on living or dead plant tissues the use of the stain known as "Pianeze IIIb" has been found very satisfactory in differentiating the fungus from the plant substratum, this differentiation occurring both in lignified and unlignified cell walls. The host tissue stains green and the mycelium a deep pink. This stain, devised by Dr. Pianeze for the study of cancer tissue,[1] is made up as follows:

Malachite green	0.50 gm.
Acid fuchsin	0.10 gm.
"Martius gelb"	0.01 gm.
Water, distilled	150.00 cc.
Alcohol, 95 per cent	50.00 cc.

Dr. Pianeze reports that it gives the following staining reactions: green in chromatin of resting or dividing nucleus, rose in cell protoplasm and membrane, and red in cancer bodies. For use with plant tissues the procedure is as follows: Wash in water or alcohol, stain in the undiluted mixture 15–45 minutes, remove excess stain in water, and decolorize in 95 per cent alcohol to which a few drops of hydrochloric acid have been added. For permanent mounts, clear with a carbol-turpentine mixture, remove clearer in xylol, and mount in balsam. Preparations of *Stereum*, *Corticium*, and *Polystictus* have been made with great success.

This stain is also valuable for staining germinated spores on the surface of a leaf. The procedure in this case is as follows: Infect marked portions of a leaf with a suspension of spores applied with a pipette, and place the plant under suitable conditions for fungous growth for 24–48 hours. Then permit

[1] Pianeze, G. Beitrag zur Histologie und Aetiologie des Carcinoms. Beiträge z. path. Anat. u. z. allg. Path. Supplement 1: 1–193. 1896. [cf. p. 58.]

the leaf to dry in the air, remove the area desired from the balance of the leaf, and place in a killing fluid. The best combined killing and tissue-clearing mixture for this purpose is one recommended by Dr. Duggar, composed of glacial acetic acid and 95 per cent alcohol. I have used equal parts of these agents most advantageously. This dissolves the chlorophyll, renders the leaf transparent or nearly so, and at the same time fixes the fungus with little plasmolysis. Allow the killing mixture to act for 24–36 hours; wash in 50 or 70 per cent alcohol, to remove the acid; and pass successively through the stain (15–30 minutes), water (2 minutes), acid alcohol (as short a time as possible), carbol-turpentine (until clear), xylol (until clearing agent is removed), and then mount in balsam. This process of differential staining has been successfully used with *Ascochyta Pisi* on pea, *Helminthosporium sativum* on barley, and *Phoma Brassicæ* on cabbage.

Pianeze's stain has not given as good results with the rusts as Durand's combination of Delafield's haematoxylin and eosin. Durand's stain[1] was not uniformly successful, however, and it was found that one of the chief difficulties often experienced finds its explanation in the killing solution which the stain follows. Flemming's solution, which was first used, gave very poor results. A modification of Gilson's mercuric chloride solution was found most satisfactory. This solution, as recommended by Dr. Durand, is made up as follows:

Water, distilled	60 cc.
Alcohol, 95 per cent	42 cc.
Acetic acid, glacial	18 cc.
Nitric acid, concentrated	2 cc.
Mercuric chloride, sat. aq. sol.	11 cc.

Diseased tissue may be fixed from 6 to 24 hours, then washed in 65 per cent alcohol, run through the alcohols, infiltrated with cedar oil, and imbedded in paraffin. This method is undesirable for nuclear structures, but gives excellent preparations for gross histological work.

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[1] Durand, E. J. The differential staining of intercellular mycelium. Phytopathology 1: 129-30. 1911.